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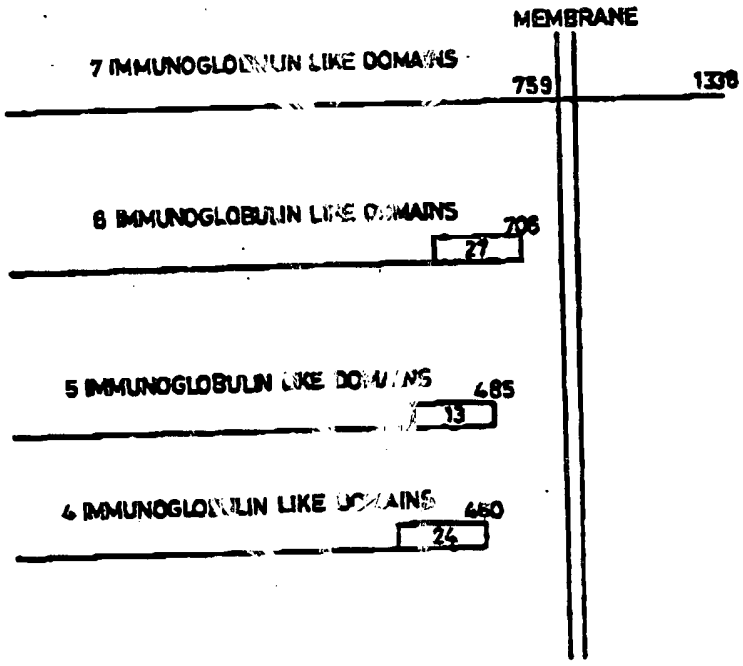

(57) Abstract

Disclosed is an altered, soluble form of the FLT polypeptide being capable of binding to VEGF and thereby exerting an inhibitory effect thereon, the polypeptide comprising five or fewer complete immunoglobulin-like domains, together with pharmaceutical compositions comprising the polypeptide, and various uses thereof.

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FLT-4 (fms-like Tyrosine kinase), FLT-15, variants thereof used as growth factor inhibitors

Field of the Invention

This invention relates to substances which inhibit growth factors, in particular, vascular endothelial growth factor (VEGF), methods of inhibiting growth factors and of treating tumours and regulating fertility.

Background of the Invention

A considerable number of human growth factors are now known, many of which have been at least partly characterised. Among them is vascular endothelial growth factor (VEGF), which has been identified in several tissues (Gospodarowicz *et al.*, 1989 PNAS *86*, 7311-7315; Conn *et al.*, 1990 PNAS *87*, 2628-2632; Tischer *et al.*, 1991 J. Biol. Chem. *266*, 11947-11954). As its name suggests, this growth factor is a highly specific mitogen for endothelial cells and is greatly involved in angiogenesis. VEGF is a homodimeric glycoprotein of two 23kDa subunits exhibiting sequence homology with platelet-derived growth factor A and B chains and placenta growth factor.

The homologous tyrosine kinase receptors fms-like tyrosine kinase receptor (FLT) and kinase insert domain-containing receptor (KDR) function as high-affinity VEGF receptors (de Vries *et al.*, 1992 Science *255*, 989-991; Terman *et al.*, 1992 Biochem. Biophys. Res. Commun. *187*, 1579-1586). Both FLT and KDR are membrane-spanning receptors that each contain seven immunoglobulin-like domains in the extracellular ligand-binding region, an intracellular tyrosine kinase domain and a transmembrane domain. The transmembrane domain serves to anchor the receptor in the cell membrane of the cells in which it is expressed.

A number of membrane-bound receptor molecules have been found to exist in truncated soluble forms, generated either by proteolytic processing or by alternative splicing of

mRNA. Recently, Kendall & Thomas (1993 PNAS *90*, 10,705-10,709, and WO94/21679) described the discovery of a soluble form of FLT receptor (sFLT) generated by alternative splicing.

Essentially, Kendall & Thomas screened a human umbilical vein endothelial cell (HUVEC) cDNA library with one probe specific for the 3' end of the flt coding region (encoding the intracellular tyrosine kinase domain) and with another probe specific for the 5' flt coding portion (encoding one of the extracellular N terminal domains). Clones which hybridised with the 5' specific probe but not with the 3' specific probe were selected for further study. In this way, a clone was isolated which encoded a soluble FLT polypeptide lacking the transmembrane domain and the intracellular domain. The truncation resulted from "readthrough" to an intronic termination codon. It was suggested by Kendall & Thomas that the soluble receptor could act as an efficient specific antagonist of VEGF *in vivo*.

The present invention is based on the discovery of further soluble variants of FLT, the existence of which was not predicted by the teaching of Kendall & Thomas.

Summary of the Invention

In a first aspect the invention provides an altered, soluble form of the FLT polypeptide being capable of binding to VEGF and thereby exerting an inhibitory effect thereon, the polypeptide comprising five or fewer complete immunoglobulin-like domains. Preferably, the altered FLT polypeptide comprises four or fewer complete Ig-like domains. The altered soluble FLT polypeptide inhibits VEGF by preventing it binding to its natural receptors, flt and KDR, present on the surface of target cells. Surprisingly, such truncated forms, lacking a major extracellular portion of the molecule, are believed to retain affinity for VEGF.

The term "soluble" as used herein is intended to refer to altered forms of the FLT polypeptide which do not comprise a transmembrane domain and thus generally do not become associated with the cell membrane of cells in which the molecule is expressed. In particular, the invention provides soluble altered forms of the FLT polypeptide

consisting substantially of four or five complete immunoglobulin-like domains.

In a particular embodiment the invention provides an altered, soluble form of FLT having at its C-terminus a region substantially having the amino acid sequence of the sequences termed FLT4 or FLT15 shown in Figure 5, or a functional equivalent thereof. The term "functional equivalent" as used above is intended to include those polypeptides which have substantially the same deletions as the polypeptides encoded by FLT4 or FLT15 (with respect to the unaltered full length FLT molecule), but which may also have other deletions, additions or substitutions, (in particular conservative substitutions), and which retain an inhibitory effect for VEGF.

Preferably the polypeptide will also comprise, at its N-terminus, the amino acid sequence substantially corresponding to the equivalent portion of the unaltered wild-type FLT polypeptide. Conveniently, polypeptides in accordance with the invention will comprise around 400 to 500 amino acid residues, preferably around 480 amino acid residues, most preferably between 480 and 440 amino acid residues of the wild type FLT sequence. Preferably the polypeptides of the invention arise by alternative splicing of mRNA or by proteolytic processing of a mature polypeptide, although it will be apparent to those skilled in the art that the polypeptide could be encoded by a nucleic acid derived, at least in part, by recombinant DNA technology.

In a further aspect the invention provides a nucleic acid sequence encoding a polypeptide in accordance with the invention. In a particular embodiment the invention provides a nucleic acid comprising the sequence of nucleotides inserted at position 1655 of the FLT 4 sequence shown in Figure 3 or the sequence of nucleotides inserted at position 1555 of the FLT 15 sequence shown in Figure 3, or a functional equivalent thereof. Examples of functionally equivalent nucleic acids include those sequences which encode substantially the same polypeptide as those encoded by FLT4 or FLT15 but which differ in nucleotide sequence as a result of the degeneracy of the genetic code. It will be apparent to those skilled in the art that the portion of the inserted nucleotide sequence in FLT4 and FLT15 occurring after the premature termination codon could be omitted without affecting the characteristics of the encoded polypeptide. Accordingly, nucleic acid molecules without

such sequences are also regarded as functionally equivalent for the purposes of the present invention.

Conveniently, the nucleic acid will substantially comprise the nucleotide sequence of FLT4 or FLT1S shown in Figure 3, together with the nucleotide sequence encoding the N-terminus of unaltered, wild-type FLT. Advantageously, the nucleic acid will be obtainable by means of PCR amplification from a sample of human cells. Desirably, the nucleic acid will be obtainable by means of PCR using primers intended to hybridise to non-conserved regions of the FLT molecule. Conveniently, the nucleic acid sequence will be obtainable by use of PCR primers designed to hybridise to the regions of the FLT sequence shown underlined in Figure 3, or immediately adjacent thereto. In particular, the PCR primers will conveniently have substantially the sequence: 5'- GCAAGGTGTGACTTTGTTC -3' and 5' - AGGATTTCTTCCCCTGTGTA -3'.

In another aspect, the invention provides a method of inhibiting VEGF *in vitro*, comprising adding an effective amount of the polypeptide defined above. It may also be desirable to inhibit VEGF in a human subject. Thus the invention provides a method of inhibiting VEGF in a human subject, comprising administeriung an effective amount of the polypeptide defined above, together with a physioologcially acceptable carrier substance. In particular, VEGF provides a mitogenic stimulus (particularly involved in angiogenesis), so inhibition of VEGF would be expected to provide therapeutic effects in the treatment of tumours or disorders involving inappropriate neovascularisation.

In particular the invention provides for a method of treating tumours or diseases involving inappropriate neovascularisation, comprising administering an effective amount of the polypeptide defined above, together with a physiologically acceptable carrier substance. Suitable diseases which might be amenable to treatment include ovarian cancer and ovarian hyperstimulation (Boocock *et al.*, 1995 J. Natl. Cancer Inst. *87*, 506-516).

Furthermore, it has been conclusively demonstrated that FLT is expressed by trophoblasts and cells from ovarian and endometrial tissues (Charnock-Jones *et al.*, 1994 Biology of Reproduction *51*, 524-530), which clearly suggests a role for VEGF in the growth and

differentiation of trophoblasts during implantation.

Thus, in particular, the invention provides a method of affecting the growth and/or migration of trophoblasts, ovarian or endometrial cells by inhibiting the action of VEGF, comprising administering an effective amount of the polypeptide defined above, together with a physiologically acceptable carrier substance.

It will be appreciated by those skilled in the art that the identification of FLT on the surface of trophoblasts and endometrial cells also provides a number of possible methods of regulating fertility. For example, the growth of trophoblasts is essential for successful implantation of the embryo. Inhibition of trophoblast growth thus provides a method of contraception or contragestion.

Thus in a further aspect the invention provides a method of regulating the fertility of a human female, comprising administering an effective amount of the polypeptide defined above, together with a physiologically acceptable carrier substance. An "effective amount" of the polypeptide is an amount sufficient to substantially block the stimulus of VEGF on trophoblasts and/or endometrial cells. Typically, the method will result in reducing the fertility of the female.

Moreover, it might be possible to identify agents which can enhance the effect of VEGF on trophoblasts, and thereby improve the probability of successful implantation, either in assisted or spontaneous cycles. Candidates for such VEGF-enhancing agents would include anti-sense equivalents of the nucleic acid sequences encoding the truncated FLT polypeptides of the invention. It will be apparent to those skilled in the art that these could be used to improve the fertility of a human female.

In a further aspect the invention provides a pharmaceutical composition comprising the polypeptide defined above, together with a physiologically acceptable carrier substance. The composition could be used in vivo any one of the methods defined above. In yet another aspect the invention provides for the use of a polypeptide in accordance with the invention in the preparation of a therapeutic composition for the treatment of tumours and

diseases involving inappropriate neovascularisation. Examples of such conditions and diseases are detailed, inter alia, in WO94/10202 and WO94/21679. The invention also includes within its scope a method of making a pharmaceutical compostion, comprising mixing the polypeptide defined above together with a physiologcially acceptable carrier substance.

The invention will now be described by way of the following illustrative examples and with reference to the drawings, of which:

Figure 1 shows an amino acid multiple alignment of closely related tyrosine kinase receptors (flt, fms and kit, "kit" being another name for KDR);

Figure 2 shows typical results of agarose gel electrophoresis demonstrating the existence of alternatively-spliced flt-coding sequences in various tissue samples;

Figure 3 shows the nucleotide sequence of the 3' region of the sequences encoding full length VEGF receptors (FLT and the related receptor KDR), together with two sequences, FLT4 and FLT15, which encode polypeptides according to the invention;

Figure 4 is a schematic representation of wild type and mutant FLT molecules; and

Figure 5 shows the C terminal amino acid sequences of two polypeptides in accordance with the invention.

Example

Expression of FLT, the VEGF receptor, was investigated in cell lines derived from human trophoblast-like and ovarian and endometrial carcinomas. The trophoblast-like (choriocarcinoma) cell line used was BeWo (obtained from the American Type Culture Collection, Rockville MD, USA). The endometrial carcinoma cell lines were Ishikawa (obtained from Professor M Nishide, University of Tsukuba, Japan), and HEC 1-A and HEC 1-B (from ATCC, USA). The ovarian cancer cell lines were 7, 17R, 25, 33R and 35. These were all shown to be of epithelial origin and had been established in culture

for 10-30 passages. Cell lines 17R and 25R were derived after chemotherapy and subsequent relapse (line 25R originating from the same patient as line 25).

BeWo cells were grown in Ham's F12, according to ATCC recommendations. Endometrial carcinoma lines were grown in McCoy's medium (ICN Flow Laboratories, Irvine, UK) with 10% foetal calf serum (ICN Flow) plus 2mM L-glutamine (ICN Flow) and 50U/ml and 50mg/ml penicillin/streptomycin (ICN Flow).

It was decided to investigate expression of FLT in these cell lines and normal tissues by performing PCT and *in situ* hybridization. It was therefore necessary to construct suitable oligonucleotide primers and probes.

To help design appropriate primers, a protein multiple alignment of closely related tyrosine kinase receptors (FLT, FMS and KIT) was constructed (shown in Figure 1) using the computer program "pileup". This revealed regions of divergent sequence among this family of receptors. The regions chosen for primer design are shown with double underlining in Figure 1. The following nested PCR primers were then synthesized based on these protein sequences:

A) 5' GCAAGGTGTGACTTTTGTTC 3'
B) 5' GCGCTCGAGAGCATCACTCAG 3'
C) 5' GCGCGGCCGCAGTAAAATCCA 3'
D) 5' AGGATTTCTTCCCCTGTGTA 3'

The underlined portions of these oligonucleotides are the regions which hybridise to the flt cDNA sequence. The other nucleotides were added to facilitate directional cloning. The cycles used were: first round with primers A and D [95°C 30 seconds, 55°C 30 seconds, 72°C 30 seconds] x 25; second round with primers B and C: [95°C 30 seconds, 44°C 30 seconds, 72°C 30 seconds] x 2 [95°C 30 seconds, 65°C 30 seconds, 72°C 30 seconds] x 25. The internal primers B and C had sites for the restriction enzymes Xho I and Eag I respectively at their 5' ends to permit directional cloning of the products.

It was found that certain tissues gave rise to PCR amplification products of notably larger size (as judged by agarose gel electrophoresis) than observed for the full length FLT cDNA product. Typical results are shown in Figure 2.

PCR products obtained using the nested set of primers A-D were run out on a gel. Lanes 1-3 are products obtained from primary tissue samples of the ovarian carcinomas designated 17, 17R and 25R. Lanes 4 to 7 are products obtained from cell lines established from the ovarian carcinomas 7, 17R, 25 and 25R. Lanes 8 to 10 are the cell lines HEC 1-A, HEC 1-B and Ishikawa respectively. Lane 11 contains products from HUVECs.

The standard size band was of the expected size (around 285bp) and was found to be identical to the 3' end of the published flt sequence (Shibuya *et al.*, 1990 Oncogene *5*, 519-524). However it can be clearly seen that in addition to the full length flt cDNA PCR-amplified product, in lanes 2 (17R, primary tissue) and 4 (7, cell line) are larger bands of approximately 360bp. A faint band of similar size was also apparent in lane 5 (17R, cell line) but is not clearly seen when the gel photograph is reproduced. These larger bands were extracted from the gel by known techniques and subcloned into the plasmid vector pBluescript II KS and then subjected to sequence analysis using the dideoxynucleotide sequencing method (Sanger et al., 1977 PNAS *71*, 5463-5467).

Sequencing of five independent clones (Boocock *et al.*, 1995 J. Natl. Cancer Inst. *87*, 506-516) revealed that each contained one of two novel insertions within the published flt sequence, in the region between the primers. Three of these clones (termed FLT5, FLT15 and FLT16) contained an 85bp insertion at about position 1555, whilst two other clones (FLT13 & FLT14) contained a 65bp insertion at about position 1665 (see Figure 3, numbering based on that of Shibuya *et al.*, 1990 cited above). The insertions account for the larger band size of the PCR products. However, both insertions contain an in-frame termination codon, so that corresponding full length RNAs would encode soluble, truncated receptor variants comprising the first five immunoglobulin-like domains of the extracellular region, up to amino acid 517 or 553, with either 24 or 14 (of which 13 are additional) unrelated amino acids at the C-terminus.

Although these variant flt clones were derived from partial cDNAs encoding only amino acids 503 onward, PCR products of the sizes predicted for corresponding full length cDNAs were amplified from cDNA derived from HUVEC cells, human chorion and ovarian carcinoma cell line 7, using primers specific for each of the novel insertions together with a primer binding just 5' of the initiating ATG (data not shown).

Figure 4 is a schematic representation of various FLT receptor molecules. At the top, (a) shows the wild type, full length FLT receptor molecule, (b) represents the truncated version described by Kendall & Thomas, (c) represents the polypeptide encoded by FLT4 and (d) represents the polypeptide encoded by FLT15. The numerals at the right show the number of amino acids in the molecule and numerals in the boxes represent the number of amino acids present in the sFLT variants but not in the wild type molecule.

Figure 5 shows the predicted C terminal amino acid sequence of the polypeptides which would be encoded by "full length" FLT4 and FLT15 clones (i.e. clones which contained all the nucleotide sequence 5' of the primer site used to generate the actual clones). The last 14 amino acids of the FLT4 clone, and the last 24 amino acids of the FLT15 clone, are divergent from the wild type FLT sequence.

Claims

1. An altered, soluble form of the FLT polypeptide being capable of binding to VEGF and thereby exerting an inhibitory effect thereon, the polypeptide comprising five or fewer complete immunoglobulin-like domains.

2. A polypeptide according to claim 1, comprising four or fewer complete immunoglobulin-like domains.

3. A polypeptide according to claim 1 or 2, having at its C terminus substantially the amino acid sequence of FLT4 as shown in Figure 5, or a functional equivalent thereof.

4. A polypeptide according to claim 1 or 2, having at its C terminus substantially the amino acid sequence of FLT15 as shown in Figure 5, or a functional equivalent thereof.

5. A polypeptide according to any one of the preceding claims, comprising around 400 to 500 amino acid residues of the wild type FLT polypeptide.

6. A nucleic acid sequence encoding a polypeptide in accordance with any one of the preceding claims.

7. A nucleic acid sequence according to claim 6, comprising the sequence of the nucleotides inserted at position 1655 of the FLT4 sequence shown in Figure 3, or a functional equivalent thereof.

8. A nucleic acid sequence according to claim 6, comprising the sequence of the nucleotides inserted at position 1555 of the FLT15 sequence shown in Figure 3, or a functional equivalent thereof.

9. A method of inhibiting VEGF *in vitro*, comprising adding an effective amount of a polypeptide in accordance with any one of claims 1 to 5.

10. A method of inhibiting VEGF in a human subject, comprising administering an effective amount of a polypeptide in accordance with any one of claims 1 to 5, together with a physiologically acceptable carrier substance.

11. A method according to claim 10, comprising the use of a polypeptide in accordance with any one of claims 1 to 5 in the treatment of tumours or diseases involving inappropriate neovascularisation.

12. A method according to claim 11, for the treatment of ovarian cancer, ovarian hyperstimulation, or endometriosis.

13. A method of affecting the growth and/or migration of trophoblasts, ovarian or endometrial cells by inhibiting the action of VEGF by administration of an effective amount of a polypeptide in accordance with any one of claims 1 to 5, together with a physiologically acceptable carrier substance.

14. A method of regulating the fertility of a human female by administration of an effective amount of a polypeptide in accordance with any one of claims 1 to 5, together with a physiologically acceptable carrier substance.

15. A pharmaceutical composition for use in the method of any one of claims 11 to 14, comprising a polypeptide in accordance with any one of claims 1 to 5, and a physiologically acceptable carrier substance.

16. A method of making a composition according to claim 15, comprising mixing a physiologically acceptable carrier substance together with a polypeptide according to any one of claims 1 to 5.

```
          1                                                  50
kit  MRGARGAWDF LCVLLLLLRV QTGSSQPSVS PGEPSPPSIH PGKSDLIVRV
fms  .........M GPGVLLLLLV ATAWHGQGIP VIEPSVP... ....ELVVKP
flt  MVSYWDTGVL LCALLSCLLL TGSSSGSKLK DPELSLKG... ...TQHIMQA

         51                                                 100
kit  GDEIRLLCTD PGFVKW.... ....TFEILD ETNENKQ... ....NEWITE
fms  GATVTLRCVG NGSVEWDGP. ....ASPHWT LYSDGSS... ....SILSTN
flt  GQTLHLQCRG EAAHKWSLPE MVSKESERLS ITKSACGRNG KQFCSTLTLN

         101                                                150
kit  KAEATNTGKY TC....T... NKHGLSNSIY VFVRDPAKLF .....LVDRS
fms  NATFQNTGTY RC....TEPG DPLGGSAAIH LYVKDPARPW ....NVLAQE
flt  TAQANHTGFY SCKYLAVPTS KKKETESAIY IFISDTGRPF VEMYSEIPEI

         151                                                200
kit  LYGKEDNDTL VRCPLTDPEV .TNYSLKGCQ GKPLPKD.LR FIPDPKAGIM
fms  VVVFEDQDAL LPCLLTDPVL EAGVSLVRVR GRPLMRH.TN YSFSPWHGFT
flt  IHMTEGRELV IPCRVTSPNI ..TVTLKKFP LDTLIPDGKR IIWDSRKGFI

         201                                                250
kit  IKSVKRAYHR LCLHCSVDQE GKSVLSEKFI LKVRPAFKAV PVVSVSKASY
fms  IHRAK.FIQS QDYQCSALMG GRKVMSISIR LKVQKVIPGP PALTLVPAEL
flt  ISNAT.YKEI GLLTCEATVN GHLYKTNYLT HRQTNTIIDV QISTPRPVKL

         251                                                300
kit  LLREGEEFTV TCTI.KDVSS SVYSTWKREN SQTKLQEK.. ..YNSWHHGD
fms  VRIRGEAAQI VCSA.SSVDV NFDVFLQHNN ..TKLAIP.. ..QQSDFHNN
flt  L..RGHTLVL NCTATTPLNT RVQMTWSYPD EKNKRASVRR RIDQSNSHAN

         301                                                350
kit  FNYERQATLT ISSARVNDSG VFMCYANNTF GSANVTTTLE VVDKGFINI.
fms  .RYQKVLTLN LDQVDFQHAG NYSCVASNVQ GKHSTSMFFR VVESAYLNL.
flt  IFYS...VLT IDKMQNKDKG LYTCRVRSGP SFKSVNTSVH IYDKAFITVK

         351                                                400
kit  FPMINTTVFV NDGENVDLIV EYEAFPKPEH QQWIYMNRTF TDKWEDYPKS
fms  SSEQNLIQEV TVGEGLNLKV MVEAYPGLQG .....FNWTY LGPFSDHQPE
flt  HRKQQVLETV AGKRSYRLSM KVKAFPSPEV V......... .....WLKD

         401                                                450
kit  ENESN..... .IRYVSELHL TRLKGTEGGT YTFLVS..NS DVNAAIAFNV
fms  PKLANATTKD TYRHTFTLSL PRLKPSEAGR YSFLAR..NP GGWRALTFEL
flt  GLPATEKSAR YLTRGYSLII KDVTEEDAGN YTILLSIKQS NVFKNLTATL
```

Fig. 1 Sheet 1

```
          451                                                        500
kit  YVNTKPEI.. LTYDRL.... ...VN..GML QCVAAGFPEP TIDWYFCPGT
fms  TLRYPPEV.. SVIWTF.... ...INGSGTL LCAASGYPQP NVTWLQCSGH
flt  IVNVKPQIYE KAVSSFPDPA LYPLGSRQIL TCTAYGIPQP TIKWFWHPCN

          501                                                        550
kit  EQRC..... .......... .......... .......... ..........
fms  TDRCD..... .......... .......... .......... ..........
flt  HNHSEARCDF CSNNEESFIL LADSNMGNRI ESITQRMAII EGKNKMASTL

          551                                                        600
kit  .......... .......... .......... .......... .....SASV
fms  .......... .......... .......... .......... .....EAQV
flt  VVADSRISGI YICIASNKVG TVGRNISFYI TDVPNGFHVN LEKMPTEGED

          601                                                        650
kit  LPV..DVQTL NSSGPPF... .......... .......... ..GKLVVQSS
fms  LQVWDDPYPE VLSQEPF... .......... .......... ..HKVTVQSL
flt  LKLSCTVNKF LYRDVTWILL RTVNNRTMHY SISKQKMAIT KEHSITLNLT

          651                                                        700
kit  IDSSAFKHNG TVECKAYNDV G.......... .......... ..........
fms  LTVETLEHNQ TYECRAHNSV G.......... .......... ..........
flt  IMNVSLQDSG TYACRARNVY TGEEILQKKE ITIRDQEAPY LLRNLSDHTV

          701                                                        750
kit  ..KTSAYFNF A.......... ..FKGNNKEQ IHPHTLFTP. ........ ..
fms  ..SGSWAF.I P.......... ..ISAGAHTH PPDEFLFTP. ..........
flt  AISSSTTLDC HANGVPEPQI TWFKNNHKIQ QEPGIILGPG SSTLFIERVT

          751                                                        800
kit  .......... .......... .......... .......LLI GFVTVAGMMC
fms  .......... .......... .......... .......VVV ACMSIMALLL
flt  EEDEGVYHCK ATNQKGSVES SAYLTVQGTS DKSNLELITL TCTCVAATLF

          801                                                        850
kit  FIVMILTYKY LQKPMYEVQW KVVEEINGNN YVYID..PTQ LPYDH.KWEF
fms  LLLLLLLYKY KQKPKYQVRW KIIESYEGNS YTFID..PTQ LPYNE.KWEF
flt  WLLLTLLIRK MKRSSSEIKT DYLSIIMDPD EVPLDEQCER LPYDASKWEF

          851                                                        900
kit  PRNRLSFGKT LGAGAFGKVV EATAYGLIKS DAAMTVAVKM LKPSAHLTER
fms  PRNNLQFGKT LGAGAFGKVV EATAFGLGKE DAVLKVAVKM LKSTAHADEK
flt  ARERLKLGKS LGRGAFGKVV QASAFGIKKS PTCRTVAVKM LKEGATASEY
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Fig. 1 Sheet 2

```
        901                                                        950
kit  EALMSELKVL SYLGNHMNIV NLLGACT.IG GPTLVITEYC CYGDLLNFLR
fms  EALMSELKIM SHLGQHENIV NLLGACT.HG GPVLVITEYC CYGDLLNFLR
flt  KALMTELKIL THIGHHLNVV NLLGACTKQG GPLMVIVEYC KYGNLSNYLK

        951                                                       1000
kit  RKRDSFI... ..C...SKQE DHAEAALYKN L......LHS KESSCSDSTN
fms  RKAEAML... ..GPSLSPGQ DPEGGVDYKN IHLEKKYVRR DSGFSSQGVD
flt  SKRDLFFLNK DAALHMEPKK EKMEPGLEQG KKPRLDSVTS SESFASSGFQ

       1001                                                       1050
kit  EYMDMKPGVS YVVPTKADKR RSVRIGSYIE RDVTPAIMED DELALDLEDL
fms  TYVEMRP... ..VSTSSN.. .....DSFSE QDLD....KE DGRPLELRDL
flt  EDKSL..... .......... .....SDVEE EEDSDGFYKE ...PITMEDL

       1051                                                       1100
kit  LSFSYQVAKG MAFLASKNCI HRDLAARNIL LTHGRITKIC DFGLARDIKN
fms  LHFSSQVAQG MAFLASKNCI HRDVAARNVL LTNGHVAKIG DFGLARDIMN
flt  ISYSFQVARG MEFLSSRKCI HRDLAARNIL LSENNVVKIC DFGLARDIYK


       1101                                                       1150
kit  DSNYVVKGNA RLPVKWMAPE SIFNCVYTFE SDVWSYGIFL WELFSLGSSP
fms  DSNYIVKGNA RLPVKWMAPE SIFDCVYTVQ SDVWSYGILL WEIFSLGLNP
flt  NPDYVRKGDT RLPLKWMAPE SIFDKIYSTK SDVWSYGVLL WEIFSLGGSP

       1151                                                       1200
kit  YPGMPVDSKF YKMIKEGFRM LSPEHAPAEM YDIMKTCWDA DPLKRPTFKQ
fms  YPGILVNSKF YKLVKDGYQM AQPAFAPKNI YSIMQACWAL EPTHRPTFQQ
flt  YPGVQMDEDF CSRLREGMRM RAPEYSTPEI YQIMLDCWHR DPKERPRFAE

       1201                                                       1250
kit  IV....QLIE KQISES.TNH I.........Y SNLANCSPNR QKPVVDHSVR
fms  IC....SFLQ EQAQEDRRER D.........Y TNLPSSSRS. .....GGSGS
flt  LVEKLGDLLQ ANVQQDGKDY IPINAILTGN SGFTYSTPAF SEDFFKESIS

       1251                                                       1300
kit  INSVGSTASS SQP......L LVHDDV.... .......... ..........
fms  SSSELEEESS SEH......L TCTEQGDIAQ PLLQPNNYQF C..........
flt  APKFNSGSSD DVRYVNAFKF MSLERIKTFE ELLPNATSMF DDYQGDSSTL

       1301                                                       1350
kit  .......... .......... .......... .......... ..........
fms  .......... .......... .......... .......... ..........
flt  LASPMLKRFT WTDSKPKASL KIDLRVTSKS KESGLSDVSR PSFCHSSCGH

       1351                                              1389
kit  .......... .......... .......... .........
fms  .......... .......... .......... .........
flt  VSEGKRRFTY DHAELERKIA CCSPPPDYNS VVLYSTPPI
```

Fig.1 Sheet 3

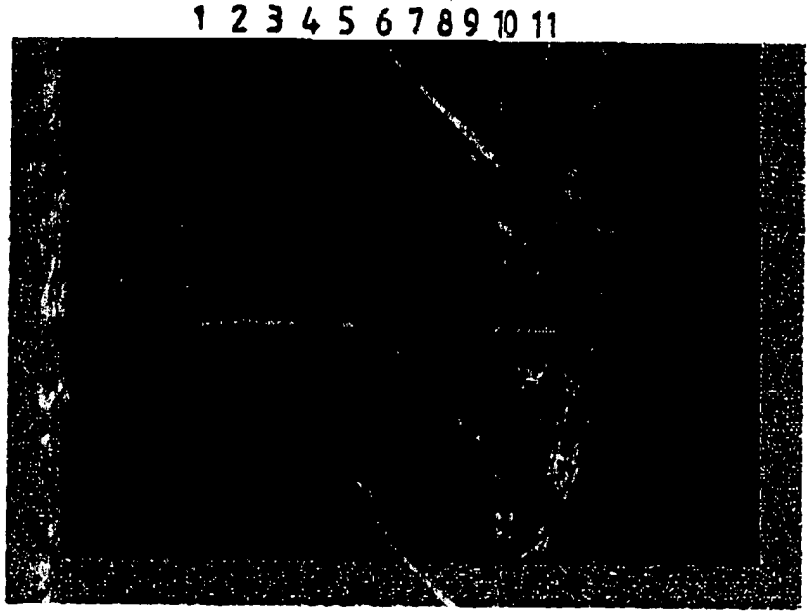

Fig. 2

```
          1410
KDR       AGAGTGCGCC AACGAGCCCA GCCAAGCTGT CTCAGTGACA AACCCATACC
FLT       ACCCCTGTAA CCATAACATT CCGAAGCAAG GTGTGACTTT TGTTCCAATA

          1460
KDR       CTTGTGAAGA ATGGAGAAGT GTGGAGGACT TCCAGGGAGG AAATAAAATT
FLT       ATGAAGAGTC CTTTATCCTG GATGCTGACA GCAACATGGG AAACAGAATT

          1510
KDR       GAAGTTAATA AAAATCAATT TGCTCTAATT GAAGGAAAAA ACAAA-----
FLT       GAGAGCATCA CTCAGCGCAT GGCAATAATA GAAGGAAAGA ATAAG-----
FLT4      GAGAGCATCA CTCAGCGCAT GGCAATAATA GAAGGAAAGA ATAAG-----
FLT15     GAGAGCATCA CTCAGCGCAT GGCAATAATA GAAGGAAAGA AGAAGCTTCC

KDR       ---------- ---------- ---------- ---------- ----------
FLT       ---------- ---------- ---------- ---------- ----------
FLT4      ---------- ---------- ---------- ---------- ----------
FLT15     ACCAGCTGAC AGTTCTTTCA TGTTGCCACC TACAAGCTTC TGTTCCAACT

                                                1555
KDR       ---------- ---------- ---------- CTGTAAGTAC CCTTGTTATC
FLT       ---------- ---------- ---------- ATGGCTAGCA CCTTGGTTGT
FLT4      ---------- ---------- ---------- ATGGCTAGCA CCTTGGTTGT
FLT15     ACTTCCATTT CCTTCCGTGA CTCTAAACGG ATGGCTAGCA CCTTGGTTGT

          1575
KDR       CAAGCGGCAA ATGTGTCAGC TTTGTACAAA TGTGAAGCGG TCAACAAAGT
FLT       GGCTGACTCT AGAATTTCTG GAATCTACAT TTGCATAGCT TCCAATAAAG
FLT4      GGCTGACTCT AGAATTTCTG GAATCTACAT TTGCATAGCT TCCAATAAAG
FLT15     GGCTGACTCT AGAATTTCTG GAATCTACAT TTGCATAGCT TCCAATAAAG

          1625
KDR       CGGGAGAGGA GAGAGGGTGA TCTCCTTCCA CGTGACCAGG ----------
FLT       TTGGGACTGT GGGAAGAAAC ATAAGCTTTT ATATCACAGA ----------
FLT4      TTGGGACTGT GGGAAGAAAC ATAAGCTTTT ATATCACAGA ATTGTCAAAC
FLT15     TTGGGACTGT GGGAAGAAAC ATAAGCTTTT ATATCACAGA ----------

KDR       ---------- ---------- ---------- ---------- ----------
FLT       ---------- ---------- ---------- ---------- ----------
FLT4      TTTGAGTGCC TTCATCCTTG CTCTCAGGAA TAGAACTCTA CCTCATCGGA
FLT15     ---------- ---------- ---------- ---------- ----------
```

Fig. 3 Sheet 1

```
                1665
KDR     -----GGTCC T---GAAATT ACTTTGCAAC CTGACATGCA GCCCACTGAG
FLT     -----TGTGC CAAATGGGTT TCATGTTAAC TTGGAAAAAA TGCCGACGGA
FLT4    TCTCATGTGC CAAATGGGTT TCATGTTAAC TTGGAAAAAA TGCCGACGGA
FLT15   -----TGTGC CAAATGGGTT TCATGTTAAC TTGGAAAAAA TGCCGACGGA

        1710
KDR     CAGGAGAGCG TGTCTTTGTG GTGCACTGCA GACAGATCTA CGTTTGAGAA
FLT     AGGAGAGGAC CTGAAACTGT CTTGCACAGT TAACAAGTTC TTATACAGAG

FLT4    AGGAGAGGAC CTGAAACTGT CTTGCACAGT TAACAAGTTC TTATACAGAG
FLT15   AGGAGAGGAC CTGAAACTGT CTTGCACAGT TAACAAGTTC TTATACAGAG

        1760
KDR     CCTCACATGG TACAAGCTTG GCCCACAGCC TCTGCCAATC CATGTGGGAG
FLT     ACGTTACTTG GATTTTACTG CGGACAGTTA ATAACAGAAC AATGCACTAC
FLT4    ACGTTACTTG GATTTTACTG CGG
FLT15   ACGTTACTTG GATTTTACTG CGG

        1810
FLT     AGTATTAGCA AGCAAAAAAT GGCCATCACT AAGGAGCACT CCATCACTCT

        1860
FLT     TAATCTTACC ATCATGAATG TTTCCCTGCA AGATTCAGGC AATTATGCCT

        1910
FLT     GCAGAGCCAG GAATGTATAC ACAGGGGAAG AAATCCTCCA GAAGAAAGAA
```

Fig. 3 Sheet 2

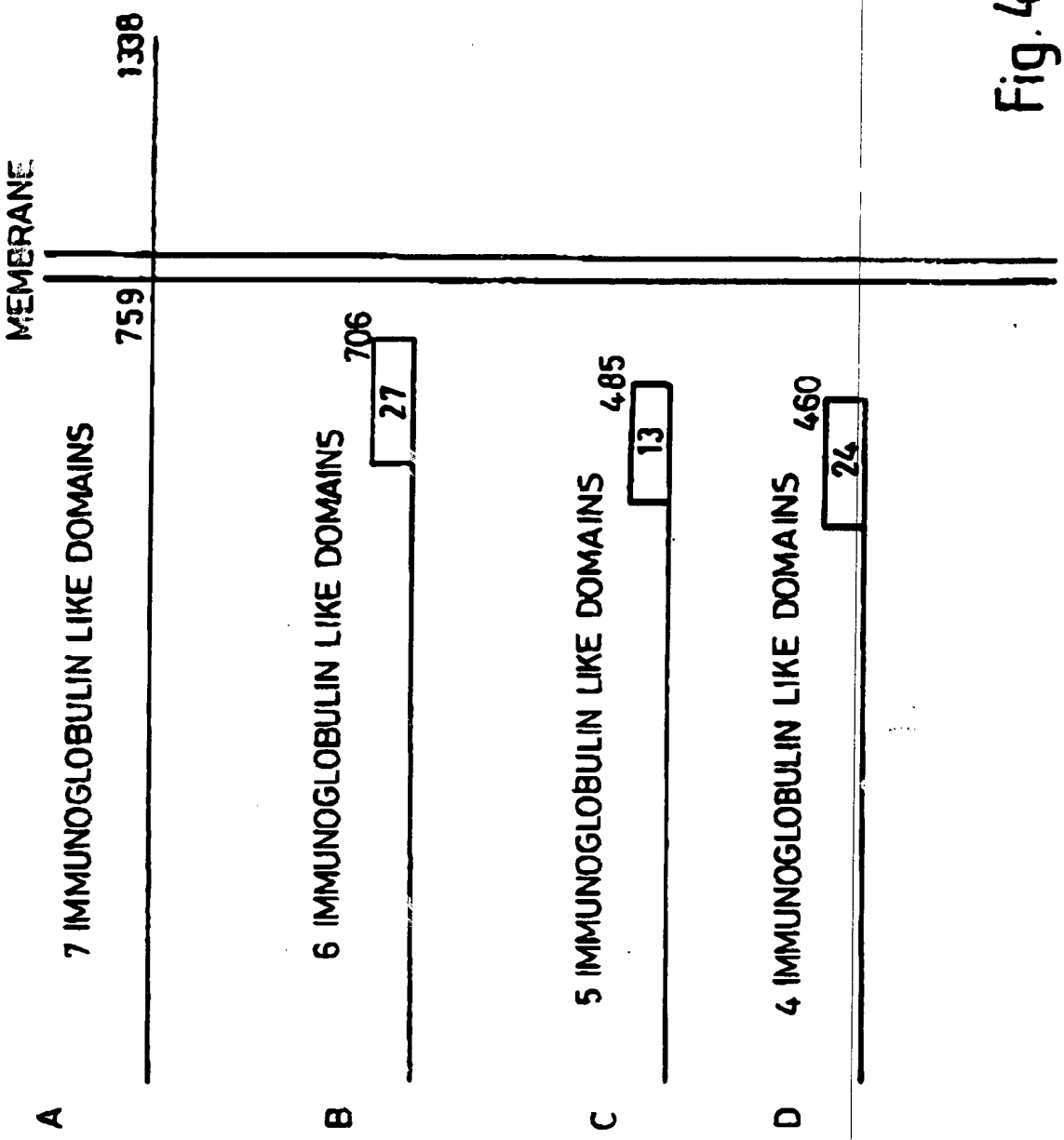
MEMBRANE
1338
759
706
27
485
13
460
24
A
7 IMMUNOGLOBULIN LIKE DOMAINS
B
6 IMMUNOGLOBULIN LIKE DOMAINS
C
5 IMMUNOGLOBULIN LIKE DOMAINS
D
4 IMMUNOGLOBULIN LIKE DOMAINS

Fig. 4

FLT4

1 ESITQRMAII EGKNKMASTL VVADSRISGI YICIASNKVG TVGRNISFYI

51 TELSNFECLH PCSQE*

FLT15

1 ESITQRMAII EGKNKLPPAD SSFMLPPTSF SSNYFHFLP*

Fig. 5

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB 95/01213

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/12 C07K14/71 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CRIT REV ONCOG, 1993, 4 (6) PS95-613, UNITED STATES, ROSNET O ET AL 'Hematopoietic receptors of class III receptor-type tyrosine kinases.' see the whole document ---	1,2,9-16
Y	ONCOGENE, vol. 8, no. 11, November 1993 ENGLAND, pages 2931-2937, PAJUSOLA, K. ET AL.; 'Two human FLT4 receptor tyrosine kinase isoforms with distinct carboxy terminal tails are produced by alternative processing of primary transcripts' see the whole document --- -/--	1,2,9-16

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
17 October 1995	08. 11. 95

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax (+31-70) 340-3016	Nauche, S

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB 95/01213

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	ONCOGENE, AUG 1993, 8 (8) P2293-8, ENGLAND, FINNERTY H ET AL 'Molecular cloning of murine FLT and FLT4.' see the whole document	1,2,9-16
Y	WO,A,94 01576 (SYSTEMIX INC) 20 January 1994 see the whole document	1,2,9-16
A	WO,A,93 15201 (NEW ENGLAND DEACONESS HOSPITAL) 5 August 1993 see the whole document	1-16
A	WO,A,92 14748 (AMERICAN CYANAMID CO) 3 September 1992 see the whole document	1-16

1

INTERNATIONAL SEARCH REPORT

Intern. ..nal application No.
PCT/GB95/01213

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 10-13
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 10-13 are directed to a method of treatment of the human/animal body as well as diagnostic methods (Rule 39.1(iv) PCT) the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB 95/01213

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9401576	20-01-94	AU-B-	4667593	31-01-94
		CA-A-	2135193	20-01-94
		EP-A-	0654088	24-05-95
WO-A-9315201	05-08-93	AU-B-	3482493	01-09-93
		CA-A-	2128722	05-08-93
		EP-A-	0624192	17-11-94
		JP-T-	7504813	01-06-95
WO-A-9214748	03-09-92	EP-A-	0536350	14-04-93